THE EFFECT OF SALINITY UPON THE RATE OF EXCYSTMENT OF ARTEMIA

R. H. JENNINGS AND D. M. WHITAKER

(*From the Department of Biology, Stanford University*)

Introduction

The phyllopod crustacean *Artemia* lives and reproduces in natural and artificial brine pools and lakes in many parts of the world. It tolerates an extreme range of salinity, pH, and other environmental conditions, although it is relatively intolerant of certain substances such as potassium [1] (Martin and Wilbur, 1921; Boone and Becking, 1931). *Artemia* does not require brine, or other environmental extremes, since it completes the life cycle in ordinary sea water in the laboratory, but it is defenseless in nature and is quickly destroyed by predators except in environments which exclude them.

Artemia are abundant in evaporating ponds on the margin of San Francisco Bay where salt is manufactured from sea water by solar evaporation. This particular *Artemia* has been regarded as a variety of *A. salina,* and also as a separate species, *A. franciscana.* Bond (1932) suggests, after experiments on the effect of salinity on development, that it should be regarded as a separate species.

The *Artemia* from the margin of San Francisco Bay reproduce by two methods. In the presence of males the same females sometimes produce viviparous nauplii, and at other times they release encysted embryos which are encased in hard chitinous coverings or shells. These encysted embryos will not ordinarily hatch in sea water until they have first been desiccated. Air-dry cysts remain viable for many years and when they are placed in sea water, the embryos hatch as swimming nauplii.

Dry cysts are approximately $\frac{1}{5}$ mm. in diameter. They are deeply indented on one side, but when they are placed in sea water, or in sea water of modified salinity in which they will hatch, they take up water and round out to become spherical. After a time, which depends among other things on salinity and temperature, the chitinous cyst wall or shell splits, and the embryo emerges head first encased within a delicate trans-

[1] This intolerance of potassium appears to be an important factor in the distribution of *Artemia* in desert salt lakes (Boone and Becking, 1931).

parent membrane or sac. This sac may remain attached at one end to the shell or it may at once be free. During the emergence from the shell and for some time thereafter the nauplius is quiescent within the sac. The sac finally begins to soften and dissolve and the nauplius moves its appendages. The nauplius completes its excystment by hatching or escaping from the remains of the sac, after which it swims actively about. At the time of hatching the nauplius contains an appreciable supply of yolk and even in the absence of food it develops for several days and undergoes the first moult to form a metanauplius. The external anatomy of the developmental stages is completely described and figured by Heath (1924).

The two stages or actions in the excystment, the initial emergence from the shell, and the final hatching from the membranous sac, will for convenience be referred to as "emergence" and "hatching" respectively. Both emergence and hatching proceed rapidly compared with the time lapse before emergence and between emergence and hatching. For accuracy in determining rates, it is necessary to define these two stages rather precisely even if somewhat arbitrarily. The emergence from the shell is a discreet abortive process and a nauplius is considered emerged if the eye can be seen. The first indication of hatching from the sac is usually the projection of the first pair of antennae. Soon the large second pair is also projected outside the sac and swimming or attempts at swimming begin. The first movements of the appendages are often intermittent and uncoördinated. A nauplius is considered hatched when the first two pairs of appendages project outside the sac and are motile. The third pair of appendages soon slides out and the remnant of the sac is left behind.

The effects of specific ions and of ion antagonism on the excystment of *Artemia* have been studied by Boone and Becking (1931), who also have concluded that osmotic pressure has much less effect on excystment than chemical factors. Jacobi and Becking (1933) observed that excystment will not take place in natural sea water concentrates of three or more molar equivalent. The present experiments were undertaken to test the effect of total salinity on the rate of excystment in diluted and concentrated sea water in which the proportion, or relative concentration, of the ions contained in sea water remains essentially unaltered. In the strongest concentration used (225 per cent sea water), there was no visible precipitation of any kind of salt so that the ionic proportions were unaltered except for second order differential effects on dissociation, and minor pH effects. The minimum salinity in which emergence, hatching, and early development will take place has also been determined.

Method

The Cysts

The cysts used in these experiments were generously provided through the courtesy of Dr. Alvin Seale of the San Francisco Aquarium Society. They were collected near Redwood City, California, June 10, 1937, and the experiments were carried out in the winter of 1939.

About 20 per cent of the original sample of cysts excysted in sea water. It was found by dissection that most of the remainder were empty shells of previously excysted embryos, although some contained embryos which were presumably dead. The empty shells are difficult to distinguish by simple inspection, but it was found that they could be partly separated out by rapid differential flotation since they contain air until they have soaked. The cysts were shaken and suspended in distilled water in a test tube and those that floated were discarded. Some good cysts were discarded by this method, and not all that sank were good cysts, but a stock was obtained in which the percentage which excysted had been increased from 20 to about 60. In this process the cysts were exposed to distilled water for only three minutes and were then dried on filter paper for five days at room temperature and 30 per cent humidity. After this they were stored for some time in a stoppered bottle before using. The brief washing in distilled water also served to remove most of the salt on the cysts, which is important for the present purpose. Otherwise, the differential flotation can be carried out as well in sea water (Whitaker, 1940). The rate of excystment in normal sea water varies with the duration of drying and storing after the washing, and would no doubt differ in different samples of cysts for this and other reasons. The present experiments were carried out on a single stock of cysts during a period in which the rate of excystment in normal sea water was practically constant.

The Media

Sea water (specific gravity, 1.025, pH 7.9–8.0) was collected at Moss Beach, California, and was filtered before being concentrated or diluted. The specific gravity 1.025 was taken as a base throughout and was considered to represent the salinity of what is called 100 per cent sea water. Sea water was diluted by adding triple glass distilled water to prepare the dilutions shown in Table I. It was concentrated by evaporating under reduced pressure in a water bath at 45–50° C. The resulting brine was diluted back with distilled water to prepare the salinities greater than sea water which are also shown in Table I. Specific gravities were

checked with pycnometers. The concentrating process removed gases from the brine so the solutions more concentrated than sea water were re-equilibrated by aerating for several hours with a sintered glass nozzle. A glass electrode was used to measure pH. The rate of excystment is practically unaffected by pH within the range 8.3–7.7, so that pH can hardly be an important factor in the present instance except perhaps in distilled water (see Table I).

Excystment

Small 1 cc. Syracuse dishes were used for excystment. Especially in the solutions of high salinity, the cysts tend to float and to accumulate in the meniscus where observation is difficult. Accumulation in the

TABLE I

Salinity and pH of media. For convenience in comparing, salinity is expressed as a percentage of the salinity of normal sea water (specific gravity 1.025), and a solution is described in terms of its relative salinity as the corresponding percentage of sea water.

Percentage Sea Water	Specific Gravity	pH
225	1.0562	8.0*
200	1.0500	8.2*
175	1.0438	8.3*
150	1.0375	8.3
125	1.0312	8.2
100	1.0250	8.0
75	1.0187	8.0
50	1.0125	7.9
25	1.0062	7.9
12½	1.0031	7.7
0†	1.0000	6.5

* Probably inaccurate due to effect of high salt concentration on glass electrode.
† Distilled water.

meniscus was prevented by dipping the dishes in hot, pure high melting point paraffin. A thin coating of paraffin causes the water meniscus to be inverted. In each experiment about twenty cysts were placed in 1 cc. of medium in each small dish, and ten small dishes were placed in Petri dishes arranged as moist chambers to prevent evaporation. The two most dilute solutions (0 and 12½ per cent sea water) were changed once in the course of the experiments so that the small amount of salt on the cysts would not appreciably alter the salinity. No measurable changes of salinity took place during the experiments. The moist chambers and the solutions were kept throughout in a humid constant temperature room at 25 ± ¼° C.

After emergence began in a population, counts were made of the numbers emerged and hatched at least every two hours until at least 60–70 per cent had hatched. The numbers that ultimately emerged and hatched were also determined at about 96 hours, and the original empty shells and non-viable cysts were excluded from consideration. The number emerged and the number hatched at the time of each observation were treated as percentages of the number that ultimately emerged and

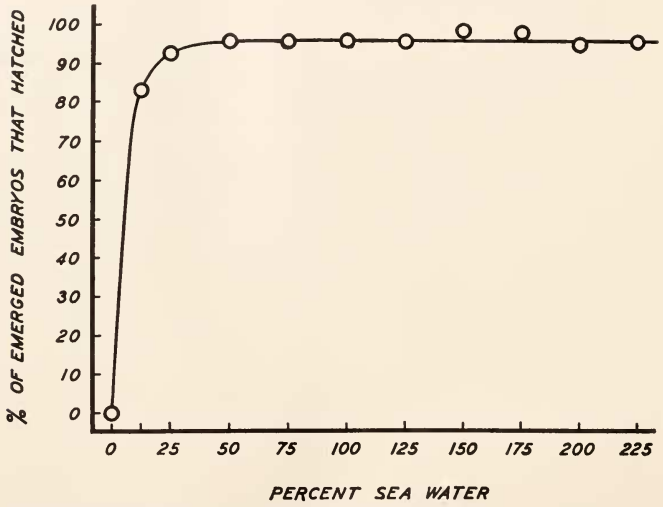

Fig. 1. The percentage of emerged embryos that hatched in diluted and concentrated sea water of various salinities. One hundred per cent sea water corresponds to specific gravity 1.025, and 0 per cent sea water is distilled water (see Table I).

hatched, respectively. The percentages obtained from successive observations were plotted against time to give signoid curves, and the times at which 50 per cent had emerged, and at which 50 per cent had hatched, were determined from these curves by interpolation.

Results

Five to nine experiments (involving counts on a total of 500–1,000 viable cysts) were carried out at each salinity. Throughout the range of salinities used, and in distilled water, approximately 60 per cent of

the stock mixture of cysts and empty shells emerged (see method), i.e. all of the embryos which are presumed to have been viable emerged in all of the solutions. More than 96 per cent of the embryos that emerged from the shell also hatched from the membranous sac in 50–225 per cent sea water, inclusive, but in lower salinities this percentage decreased and no true hatching at all occurred in distilled water (Fig. 1). In distilled water the appendages of the emerged embryos did not move. The membranous sac disintegrated after several hours in about one-fifth of

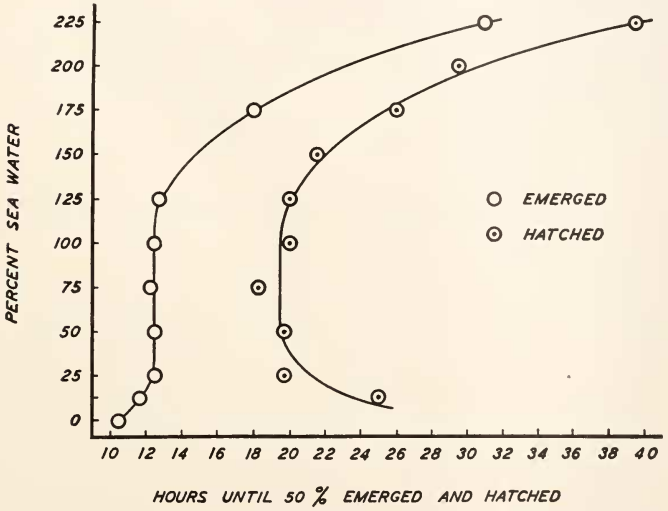

Fig. 2. The rates of emerging and hatching in diluted and concentrated sea water at 25° C. One hundred per cent sea water corresponds to specific gravity 1.025, and 0 per cent sea water is distilled water (see Table I). The curves show the time lapse until 50 per cent of the embryos in populations emerge, and until 50 per cent hatch (see text).

the cases, causing a sort of pseudo-hatching, but in these cases the nauplii always swelled, and often burst near the first joints of the large second antennae. Inactivity of the embryo probably interferes with hatching. Occasional cysts burst within 20–30 minutes after being placed in distilled water and aborted amorphous masses. This also occurred rarely in 12½ per cent sea water and in higher salinities as well.

The embryos that hatched throughout the range 12½–225 per cent

sea water were all normal, active, and viable in the salinity in which they excysted. They moulted once before dying of starvation on about the fourth day. No food was provided and no attempt was made to determine the salinity requirements of more advanced developmental stages. The effect of salinity on food organisms is a complicating factor after the yolk has been consumed.

The effect of salinity on the rate of emergence and hatching is shown in Fig. 2. Each point represents the average of the recorded time lapses until 50 per cent had emerged and until 50 per cent had hatched in the several experiments at each salinity. The results of the individual experiments were quite consistent. It may be seen in Fig. 2 that the rates of emergence and hatching are little affected by osmotic pressure within the salinity range 25–125 per cent sea water, but this is not true in higher and lower salinity.

Summary and Conclusions

1. The excystment of *Artemia* takes place in two principal stages: first, the quiescent nauplius emerges from the shell of the cyst within a membranous sac, and then later the nauplius hatches from the sac and swims actively about.

2. The excystment of *Artemia* obtained from the margin of San Francisco Bay has been studied at 25° C. in diluted and concentrated sea water over a salinity range from zero (distilled water) to 225 per cent sea water (i.e., a solution in which the salt concentration is 225 per cent of the salt concentration of sea water. No salts precipitated out).

3. The same percentage of embryos emerged from the shells in all of these salinities, including zero salinity (distilled water).

4. In distilled water the emerged embryos are motionless and they do not hatch from the sac. Some swell and burst.

5. In 12½ per cent sea water, 83 per cent of the emerged embryos hatch; in 25 per cent sea water 93 per cent hatch. In 50–225 per cent sea water 96–99 per cent hatch.

6. In 12½–225 per cent sea water the nauplii that hatch are normal, active, and viable. They moult to form metanauplii before dying of starvation (in the absence of food) on about the fourth day.

7. In the salinity range 25–125 per cent sea water, the rates of emergence and hatching are practically constant (and therefore independent of change in osmotic pressure).

8. In the salinity range 150–225 per cent sea water the rates of emerging and hatching decrease with increasing salinity, but the interval between emergence and hatching is nearly constant throughout the range 25–225 per cent sea water.

9. Emergence is accelerated in 12½ and 0 per cent sea water, but hatching is retarded in 12½ per cent sea water and is inhibited in distilled water.

BIBLIOGRAPHY

Bond, R. M., 1932. Observations on Artemia "franciscana" Kellogg, especially on the relation of environment to morphology. *Int. Rev. der. ges. Hydrobiol. und Hydrogr.*, **28**: 117–125.

Boone, E., and L. G. M. Baas-Becking, 1931. Salt effects on eggs and nauplii of Artemia salina L. *Jour. Gen. Physiol.*, **14**: 753–763.

Heath, H., 1924. The external development of certain phyllopods. *Jour. Morph.*, **38**: 453–483.

Jacobi, E. F., and L. G. M. Baas-Becking, 1933. Salt antagonism and effect of concentration in nauplii of Artemia salina L. *Communications from the Leiden Botanical Laboratory*, No. 2.

Martin, E. G., and B. C. Wilbur, 1921. Salt antagonism in Artemia. *Am. Jour. Physiol.*, **55**: 290–291.

Whitaker, D. M., 1940. The tolerance of *Artemia* cysts for cold and high vacuum. *Jour. Exper. Zoöl.*, **83**: 391–399.